STAT1-deficient T cells were indeed deficient in Th1 cells post-BMT. Relative to recipients of WT T cells, recipients of STAT1-KO T cells had reduced capacity to secrete IFN-γ (4661±664 vs. 2656±281; $p < 0.03$) and TNF-α (3154±269 vs. 1872±84; $p < 0.001$) and reduced absolute numbers of splenic CD4+IFN-γ+ T cells (14±1.9 vs .07±.02; $p < 0.05$) and CD8+IFN-γ+ T cells (4.1±1.2 vs. 0.8±.1; $p < 0.05$). As such, recipients of STAT1-deficient T cells were indeed deficient in Th1-polarization post-BMT during cGVHD. Remarkably, relative to WT T cell recipients, recipients of STAT-1 deficient T cells had increased absolute numbers of post-BMT splenic CD4+IL-17± T cells (0.2±0.05 vs 13.2±6.5.; $p < 0.05$). These data indicate that long-term post-BMT deficiency of Th1-type cells does not protect against chronic GVHD, perhaps in part due to expansion of alternative pathogenic T cells such as the Th17 subset.

500

THE ROLE OF COMPLEMENT SYSTEM IN THE PATHOGENESIS OF GRAFT VERSUS HOST DISEASE

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Introduction: Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in bone marrow transplant (BMT) recipients. GVHD is classically described as T-cell lymphocytic infiltration followed by destruction of tissue caused by differences in HLA. However, treatments aimed at other components of the immune system have been successful, questioning this paradigm. The role of the complement system in GVHD is limited. In solid tumor transplantation, activation of the complement system and link to preformed anti-HLA antibodies in recipients has been associated with rejection. We describe the evaluation of complement activation in 53 patients with clinical GVHD in whom 40 specimens of skin and 7/13 specimens of colon showed evidence of GVHD by conventional histochemical staining method. In addition, we analyzed 11 control patients with normal colon biopsies. Analysis of complement fixation was performed using C4d antibody staining method and was analyzed using the Banff07 grading system where minimal staining is denoted as positive in < 10% of vessels and diffuse staining is positive in > 50% of vessels. Histology of GVHD was correlated with C4d deposition. Statistical analysis using Fisher's exact test was performed between groups and with controls.

Results: Thirty-four of forty skin biopsies were evaluable for C4d deposition (6 specimens showing extensive background deposition were excluded). Twenty-one of thirty-four specimens showed C4d staining (11 minimal, 9 focal, and 1 diffuse). Twelve of the thirteen colon biopsies showed C4d staining (2 minimal, 4 focal, and 6 diffuse). Of the 11 control colon biopsies, 10 were negative for C4d and 1 showed minimal staining. In the cohort that showed clinical GVHD not pathologically confirmed, 6/6 showed C4d staining. The difference in C4d staining between the GVHD of the colon and colon control specimens was statistically significant using a Fisher's exact test (p-value = 0.00000177). In addition, there was statistical difference between Cd4 staining in clinical GVHD vs. controls with a p value of 0.0000127.

Conclusion: Our results demonstrate that most patients with clinical GVHD showed vascular staining for C4d. Clinical GVHD and C4d deposition were closely correlated and statistically different from controls. This may indicate that C4d positivity is a more sensitive marker in detecting GVHD of the colon than classic GVHD histological evaluation.

501

SUSTAINED HIGH-DOSE CORTICOSTEROID USE DOES NOT INDEPENDENTLY DIMINISH SURVIVAL AFTER MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction: Corticosteroids remain the most efficacious treatment for acute GVHD (aGVHD) following allogeneic hematopoietic stem cell transplantation (AlloHSCT). Some patients with aGVHD receive high-dose (HD) corticosteroids for ≥ 2 months for a variety of reasons. Morbidities related to long term corticosteroid use are well recognized, however, the impact of dosing and treatment duration on survival has not been fully evaluated in the post-AlloHSCT setting.

Methods: From 1998 to 2009, 272 patients with AlloHSCT were treated with corticosteroids between post-transplant days 30 and 100 at a single academic institution. Patients in HD group received ≥ 30 mg prednisone daily for ≥ 2 months, whereas those in the low-dose (LD) corticosteroid group received < 30 mg at some point in this interval or ≥ 30 mg for < 2 months. Relapse-free (RFS) and overall survival (OS) were compared between corticosteroid groups. Cox proportional hazards analysis identified prognostic factors.

Results: HD (n = 98) and LD (n = 174) groups were similar in distribution of demographic characteristics, comorbidity index, diagnosis, number of previous chemotherapies and conditioning regimen (all $p \geq 0.3$). HD group had more MUD AlloHSCT ($p = 0.05$) and higher-grade aGVHD ($p < 0.001$). Malignancy relapse in HD and LD groups was 24% and 36%, whereas overall mortality was 59% and 55% respectively. Non-relapse mortality tended to be higher (HR = 1.5; 95% CI, 1.0-2.3) in HD group. No significant association was detected between the HD corticosteroid use and infection-related mortality ($p = 0.1$), relapse mortality ($p = 0.2$), RFS ($p = 0.8$), or OS ($p = 0.4$). Multivariable analysis of non-relapse mortality demonstrated no independent prognostic significance of HD corticosteroid use (HR = 1.3; 95% CI, 0.8-2) after adjustment for comorbidity index (HR = 2 for high vs. low HCT-CI; 95% CI, 0.8-2), source of hematopoietic stem cells (HR = 2.3 for peripheral vs. bone marrow; 95% CI, 1.3-4.1) and grade of aGVHD (HR = 1.3 per 1 grade increase; 95% CI, 1.1-1.6).

Conclusions: Using HD corticosteroids for ≥ 2 months did not appear to have an independent impact on patient survival following AlloHSCT and therefore it may be safe to use as necessary for aGVHD control. The association of HD steroids with non relapse mortality appears to be related to worse aGVHD.

502

THYMIC IRRADIATION IS REQUIRED FOR TRANSPLANTATION TOLERANCE AFTER TLI/ATS NON-MYELOABLATIVE CONDITIONING

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Total Lymphoid Irradiation and Anti-Thymocyte Serum (TLI/ATS) conditioning induces potent donor-host tolerance and GVHD protection via induction of donor $CD4^+CD25^+Foxp3^+$ Treg after bone marrow transplantation (BMT) (Pillai et al, Blood 2009). Whereas total body irradiation (TBI) at doses as low as 400 cGy associate with lethal acute GVHD after MHC-mismatched BMT, TLI exposes GVHD target organs to a cumulative radiation dose of 2400 to 4080 cGy, without GVHD. We investigated the role of radiation exposure to specific anatomic areas in tolerance induction after TLI/ATS + BMT. Wild-type (WT) BALB/c ($H\text{-}2^d$) hosts received infusion of 50 x 10^6 bone marrow + 60 x 10^6 splenocytes from WT C57BL/6 ($H\text{-}2^b$) donors (BMT) following 5 doses of rabbit ATS + 10 doses (240 cGy each2400 cGy total) of fractionated TBI, TLI, or TLI with lead shielding of specific regions, including TLI with focal thymic shielding (TLI-FTS). Day 6 $H\text{-}2K^{b+}CD8^+$ and $H\text{-}2K^{b+}CD4^+CD25^{neg}$ effector cell and $H\text{-}2K^{b+}CD4^+Foxp3^+$ Treg accumulation was quantified in target organs, and GVHD scoring performed on histopathologic sections. Positive controls included WT BALB/c hosts receiving ATS + single-dose 800cGy TBI (PC-1) or ATS + 400 cGy TBI (PC-2) and 50 x 10^6 bone marrow + 60 x 10^6 splenocytes from C57BL/6 donors. Negative controls received 800cGy TBI and 50 x 10^6 bone marrow from WT C57BL/6 (NC-1) or ATS alone + BMT (NC-2). All fractionated TBI hosts died during conditioning with histopathologic systemic radiation toxicity. In TLI/ATS hosts at day 6 after BMT, accumulation of $H\text{-}2K^{b+}TCR^+CD8^+$ cells was strongly suppressed in colon, MLN, and spleen ($p < 0.01$), with increased accumulation of donor Treg ($p < 0.01$) compared to PC-1 controls. Mean colonic